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Synthesis and Evaluation of new Ethyl N-[(Z)-(2-Oxo-5-Sulfamoyl-Indolin-3-Ylidene) Amino] Carbamate Derivatives for their Antimicrobial and Anti-inflammatory Activity

Srikanth Lingala[1*], Kiran Samudrala[1], Raghunandan Nerella[1]
[1]Department of Pharmaceutical Chemistry, Balaji Institute of Pharmaceutical Sciences, Warangal, India.

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ABSTRACT

In view of various biological activities and enormous importance of indoles, isatins and their derivatives, it was our interest to synthesize and characterize some new 5-Sulfamoyl Isatin derivatives and evaluate them for antimicrobial and anti-inflammatory activity. An appropriate quantity of isatin hydrazone was heated under reflux with ethylchloroformate to give ethyl n-[(z)-(2-oxo-5-sulfamoyl-indolin-3-ylidene)amino]carbamate which was then heated under reflux with various primary amines to give ethyl n-[(z)-(2-oxo-5-sulfamoyl-indolin-3-ylidene) amino]carbamate derivatives. The intermediates and final compounds were purified and their chemical structures have been confirmed by IR, ^{1}H NMR, and Mass spectral data. All the synthesized compounds were screened for antibacterial activity against *B. subtilis, B.cereus, S. epidermidis, S. typhi, P. aeruginosa* and *K. pneumoniae*, antifungal activity against *A. flavus, F. oxysporium* and *P. notatum* and anti-inflammatory activity using carrageenan induced rat paw edema model. Most of the compounds tested have shown promising activities when compared with the standard drugs.

INTRODUCTION

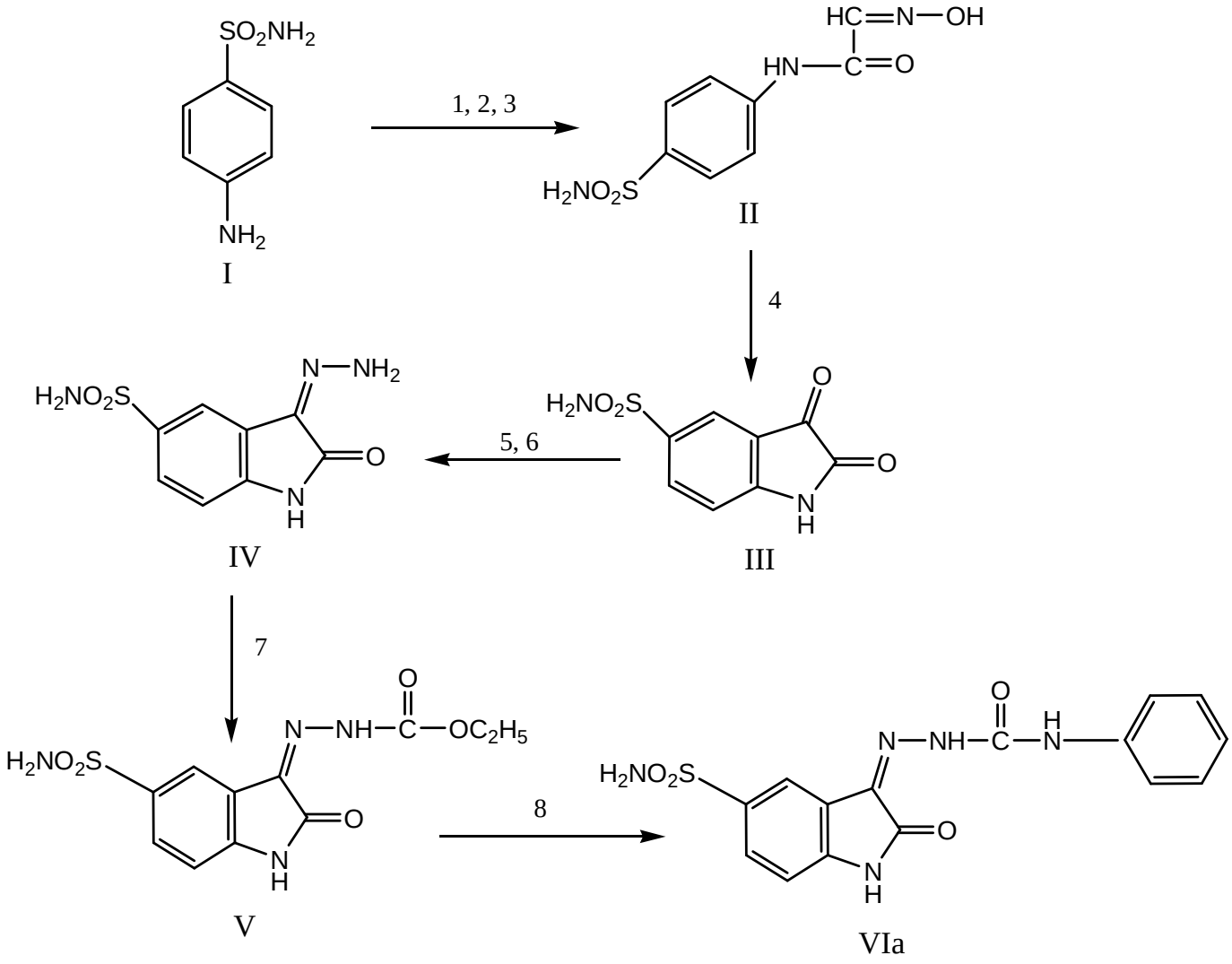
Isatins are an important group of heterocyclic compounds which are biologically active and of significant importance in medicinal chemistry. A variety of biological activities are associated with isatin including CNS activities as potentiation of pentobarbitone induced necrosis, analgesic, anticonvulsant, antidepressant, anti-inflammatory, antimicrobial and effects on the central nervous system (Harish, 2009). Isatins are capable of crossing the blood–brain-barrier (Durell and Pollin, 1963). Isatin, a heterocyclic compound was identified in animals as a major component of the endogenous monoamine oxidase inhibitor (Bhanupriya *et al*., 2010). Isatin (1H-indole-2,3-dione) is a synthetically versatile substrate, where it can be used for the synthesis of a large variety of heterocyclic compounds, such as indoles and quinolines, and as a raw material for drug synthesis. Isatin has also been found in mammalian tissues, and its function as a modulator of biochemical processes has been the subject of several discussions.

* *Corresponding Author*
Srikanth Lingala, Email:srikanth802@gmail.com
Phone:+91 9492686402; 08718 230521

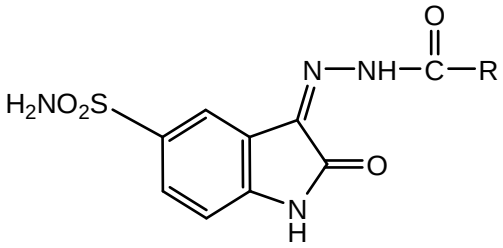
A survey of literature reveals the advances in the use of isatin for organic synthesis during the last twenty-five years, as well as enormous importance of its biological and pharmacological properties. In view of these valid observations in our present study, we reported the synthesis of new 5-Sulfamoyl Isatin derivatives and the synthesized compounds were screened for their antibacterial, antifungal and anti inflammatory activity.

MATERIALS AND METHODS

The chemicals and solvents used for the experimental work were commercially procured from E. Merck, India, S.D. Fine Chem, India and Qualigens, India. Silica gel G used for analytical chromatography (TLC) was obtained from S.D. Fine Chem, India. Melting points were determined in an open glass capillary using a Kjeldahl flask containing liquid paraffin and are uncorrected. The proton magnetic resonance spectra (^{1}H NMR) were recorded on a Bruker 300 MHz instrument (Bruker, Germany) in DMSO/$CDCl_3$ using TMS as internal standard. Chemical shifts (δ) are expressed in ppm. The infrared spectra of compounds were recorded in KBr on a FTIR- -8400S, Fourier Transform (Shimadzu), Japan infrared spectrophotometer. Mass spectra were recorded on LC-MS/MS (API-4000 TM), Applied Bio Systems, MDS SCIEX (Canada).

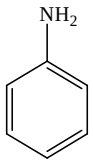
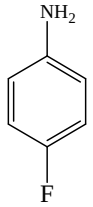
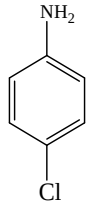
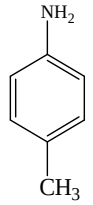
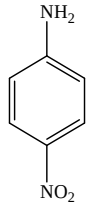


1. Chloralhydrate
2. Hydroxylamine hydrochloride
3. Sodium sulphate
4. Conc. Sulphuric acid
5. Absolute Alcohol
6. Hydrazinehydrate
7. Ethylchloroformate
8. Aniline (Various primary amines)



SCHEME. 1:

Table. 1: Physical data of synthesized compounds (VIa - VIe).

Comp. no.	R	Molecular formula	Mol. Wt.	% yield	Melting Point (°C)	R_f Value
VIa		$C_{15}H_{13}N_5O_4S$	359.36	75%	192 - 194	0.73
VIb		$C_{15}H_{12}N_5O_4SF$	377.35	69%	198 - 201	0.69
VIc		$C_{15}H_{12}N_5O_4SCl$	393.80	81%	209 - 212	0.44
VId		$C_{16}H_{15}N_5O_4S$	373.39	79%	220 - 223	0.64
VIe		$C_{15}H_{12}N_6O_6S$	404.36	72%	206 - 208	0.58

Solvent system used for TLC: Pet.ether : Ethylaceteate (3:2)

Table. 2: Antibacterial activity of synthesized derivatives. (VIa - VIe).

Comp.	Zone of inhibition (mm)					
	B.subtilis	***B.cereus***	***S.epidermidis***	***S.typhi***	***P.aeruginosa***	***K.pneumoniae***
VIa	9.1	6.3	10.3	9.7	10	8.1
VIb	11	12.1	11.4	12.8	14.6	11
VIc	12.2	14.2	12.4	13.3	13.9	8.4
VId	6.3	7.4	10.2	7.9	11.4	5.2
VIe	14.6	16.2	18.4	17.8	18.2	13.6
Negative Ctrl.	--	--	--	--	--	--
Standard (Amikacin)	22	19	20	22	20	18

-- *No activity, Negative Control – DMSO*

Table. 3: Antifungal activity of synthesized derivatives. (VIa - VIe).

Comp.	Zone of inhibition in(mm) (200µg/100µl)		
	A.flavus	***F.oxysporium***	***P.notatum***
VIa	4	--	6
VIb	--	11.2	11.8
VIc	7.4	5.2	4.2
VId	--	--	5.8
VIe	13.8	12.9	11.5
Negative Control(DMF)	--	--	--
Standard (Amphotericin B)	19	16	19

-- *No activity; the antifungal activity was assayed by cup plate method; 100 µl/cup (disc diameter is 6mm) Amphotericin B (100µl/cup) was used as +ve ctrl.*

EXPERIMENTAL

Synthesis of 5-Sulphamoyl Isonitrosoacetanilide (II)

General procedure

5gm of Sulfanilamide was taken and added to 30 ml of Distilled water and 5ml of concentrated Hydrochloric acid (HCl), which gave a clear solution in the beaker. (solution I). In another beaker 9gms of Chloral hydrate and 30 ml of water was taken and this solution was made up to 150ml. (solution II). The solution I and II were mixed. To this mixed solution anhydrous Sodium Sulphate (Na_2SO_4) was added and the solution was stirred until precipitate was formed. 12gms of Hydroxylamine Hydrochloride was taken and added to 150ml of water (Solution III).

The above the solutions were mixed and the solution was heated on a water bath for 45 minutes and kept side for 12-24 hours (Ankur Patel *et al*., 2006) to give compound II. M.P : 170-173 ^oC, Yield : 75%.

Synthesis of 5-Sulfamoyl-1H-Indole-2,3-dione (III)

General procedure

1 gram the above crystals, was taken in dry beaker and 4ml of concentrated Sulphuric acid was added by stirring and the temperature was maintained at 60 ^oC. The beaker was kept aside for 12 hrs then this solution was poured into the crushed ice to give 5-Sulfamoyl-1H-Indole-2,3-dione as a coloured precipitate (Marvel, 1941). M.P : 198-201 ^oC, Yield: 70%.

Synthesis of 5-Sulfamoyl isatin hydrazones (IV)

An appropriate quantity of isatin **(III)** (0.01 mol) was dissolved in alcohol (20ml) and hydrazinhydrate (0.01 mol) with shaking. The reaction mixture was stirred well, refluxed for 3 hours. The resultant crystalline solid was filtered, washed repeatedly with small portions of cold water and finally with a small portions of alcohol (Srikanth *et al*., 2010). The product was dried and purified by recrystallization from chloroform. M.P : 198 ^oC, Yield: 68%. **IR (KBr)(cm^{-1}):** 3250(N-H str.), 3155 (Ar-H str.), 1650-1540 (C=C & C=N str.), 1345, 1160 (SO_2), 1240 (C-N). **^{1}H NMR (DMSOd_6): δ** 7.2 (s, 2H, N-NH_2), 2.3 (s, 2H, S-NH_2), 10 (s, 1H, -NH), 7.8 – 8.2 (d, s, 3H, Ar-H). **EI-MS: *m/z*** = 240(M^+).

Synthesis of Ethyl N-[(Z)-(2-oxo-5-sulfamoyl-indolin-3-ylidene)amino]carbamate (V)

An appropriate quantity of isatin hydrazone **(IV)** (0.01mol) was heated under reflux with Ethylchloroformate (0.01mol) in dry acetone under anhydrous conditions using calcium chloride guard tube for 2 hrs. The product thus formed was filtered and washed with small portions of acetone to remove any unreacted Ethylchloroformate (Vivek *et al*., 2010) . It was purified by recrystallization with ethanol. M.P; 182 ^oC Yield; 74 %. **IR (KBr)(cm^{-1}):** 3230(N-H str.), 3145 (Ar-H str.), 1645-1555 (C=C & C=N str.), 1342, 1745 (C=O str.), 1240 (C-O), 1163 (SO_2), 1268 (C-N). **^{1}H NMR (DMSOd_6): δ** 7.2 (s, 1H, N-NH-C), 2.3 (s, 2H, S-NH_2), 10.1 (s, 1H, -NH), 7.6 – 8.2 (d, s, 3H, Ar-H), 4.3 (q, 2H, -CH_2-), 3.5(t, 3H, -CH_3). **EI-MS: *m/z*** = 312(M^+).

Synthesis of 1-[(2-oxo-5-sulfamoyl-indolin-3-ylidene)amino]-3-phenyl-urea (VIa)

Ethyl N-[(Z)-(2-oxo-5-sulfamoyl-indolin-3-ylidene) amino]carbamate **(V)** (0.01mol) was heated under reflux with various primary amines (aniline) (0.01mol) in dry acetone under anhydrous conditions using calcium chloride guard tube for 2 hrs. The product thus formed was filtered and washed with small portions of acetone to remove any unreacted primary amines it was purified by recrystallization with ethanol (Srikanth *et al*., 2011) . The physical data of all the newly synthesized derivatives is given in Table 1. M.P; 192 – 194 ^oC Yield; 75 %.

IR (KBr)(cm^{-1})

3236 (N-H str.), 3142 (Ar-H str.), 1640-1556 (C=C & C=N str.), 1338, 1166 (SO_2), 1247 (C-N). **^{1}H NMR (DMSOd_6): δ** 7.0 (s, 1H, N-NH-C), 6.2 (s, 1H, CO-NH-C), 2.0 (s, 2H, S-NH_2), 9.8 (s, 1H, -NH), 7.4 - 8.5 (8H, m, Ar-H). **EI-MS: *m/z*** = 359(M^+), 360(M+1).

BIOLOGICAL EVALUATION

Antibacterial Activity

Antibacterial activity of the synthesized compounds was determined, using a slightly modified cup plate method (Ansari and Lal, 2009) Muller Hinton agar was used for the growth of bacterial strains (*B.subtilis* (MTCC 121), *B.cereus* (ATCC 14579), *S.epidermidis* (ATCC 25923), *S.typhi* (MTCC 733), *P.aeruginosa* (MTCC 741) and *K.pneumoniae* (ATCC 29212). Each organism was suspended in normal saline solution and transmittance (T) of 75 to 77% at 530 nm was made, which is equal to 10^6 CFU/ml. All the test compounds were dissolved in DMSO at a concentration of 2 mg/ml. Each plate was inoculated with 20 µl of microbial suspension. 100 µl of the test compounds was added to each cup. The plates containing bacteria were incubated at 37^o C for 24 hrs, the positive antimicrobial activity were read based on the growth inhibition zone and compared with the solvent as a negative control and Amikacin as comparative drug, and the results are presented in the Table 2. All the synthesized compounds have shown antibacterial activity.

ANTIFUNGAL ACTIVITY

The medium was prepared by dissolving all the ingredients in distilled water and subjected to sterilization in an autoclave at 121 ^oC /15lbs for 15 minutes. The Petri plates were washed thoroughly and sterilized in hot air oven at 160 ^oC for 1 ½ hours. 30 ml of sterile SDA was seeded by organisms (about 2 ml according to Mc Farland's standard), in semi hot conditions (40^oC) was poured aseptically in sterile Petri plate and allowed to solidify at room temperature. Bores were made on the medium using sterile borer and 0.1 ml of the solution of synthesized compounds at 2mg/ml concentration in DMF were added to respective bores and 0.1ml of the standard Ampotericin B at a concentration of 200 µg/0.1ml was used as standard as shown in Table 3. The Petri

plates seeded with fungal organisms, containing solution of synthesized compounds and the standard drug were kept in a refrigerator at 4 ^{o}C for 1 hour to facilitate the diffusion of the compounds and the standard in to the media. After diffusion the Petri plates were incubated at 28 ^{o}C for one week and later the zone of inhibition was observed and measured using a scale (Ayhan-Kilcigil and Altanlar, 2003) and the results are presented in the Table 3.

ANTI-INFLAMMATORY ACTIVITY

Wistar strain albino rats weighing between 180-250 gm fasted 24 hrs before the test, were divided into four groups of six animals each. The volume of the right hind paw was measured using a plethysmometer. This constituted the initial reading compounds were tested in the dose of 50 mg/kg body weight. Diclofenac 20 mg/kg was used as standard. The compounds were administered as suspensions in sodium CMC (0.1% w/v) intraperitoneally 30 min before the injection of carrageenan. Control group of animals received a suspension of sodium CMC only 0.1ml of 1.0% w/v carrageenan suspension in normal saline was injected into the plantar region of the right hind paw. The swelling produced after injection of the phlogistic agent was measured (Bahaa *et al*., 2006) at hourly intervals for 6 hrs. Percentage inhibition of edema was calculated using the formula given below and the results are presented in the Table 4.

$$\% \text{ inhibiti on of edema} = \frac{\text{Mean edema of control group-mean edema of treated group X 100}}{\text{-mean edema of control group}}$$

RESULTS AND DISCUSSION

From the antibacterial screening it was observed that all the compounds exhibited activity against all the organisms employed as indicated in Table 2. The compound **VIe** has shown good activity against both the Gram-positive and Gram-negative bacteria. The compound showed maximum zone of inhibition (18.4 mm) against *S.epidermidis* and zone of inhibition (18.2 mm) against *P.aeruginosa* Compounds **VIc** and **VIb** have shown good antibacterial activity, compound **VIc** has shown zone of inhibition (14.2 mm) against *B.cereus* and zone of inhibition (13.9 mm) against *P.aeruginosa*. The compound **VIb** has shown moderate activity against all the organisms. Compound **VId** has shown less activity among all the synthesized compounds followed by compound **VIa**. But all the derivatives have shown less antibacterial activity when compared to the standard drug Amikacin.

The antifungal activity of the compounds studied against *A.flavus, F.oxysporium and P.notatum* is shown in Table 3**.** Amphotericin B was used as reference for inhibitory activity against fungi. It was observed that the compound **VIe** showed maximum zone of inhibition, 13.8mm against *A.flavus* and zone of inhibition of 12.9 mm and 11.5 mm against *F.oxysporium* and *P.notatum*. Compound **VIb** has shown good activity against *F.oxysporium and P.notatum* with zone of inhibition, 11.2 mm and 11.8 mm, but the compound **VIb** has not shown any activity against *A.flavus*. The compound **VIc** has shown moderate activity against all the three strains employed for the study. The compound **VIa** has shown mild activity on *A.flavus* and *P.notatum* with zone of inhibition of 4 mm and 6 mm, but the compound **VIa** has not shown any activity against *F.oxysporium*. The compound **VId** has shown mild activity only on *P.notatum* with zone of inhibition of 5.8 mm, compound **VId** has not shown any activity against *A.flavus* and *F.oxysporium*.

The preliminary studies on anti-inflammatory activity of the new title compounds ie., ethyl n-[(z)-(2-oxo-5-sulfamoyl-indolin-3-ylidene)amino]carbamate derivatives (VIa - VIe) generated some interesting data. The test compounds were evaluated for anti-inflammatory activity and data are presented in Table 4 using diclofenac sodium (20 mg/kg) as the standard.

The close observation of anti-inflammatory activity of all the test compounds shows that all the test compounds showed mild to moderate anti-inflammatory activity. Compounds (**VIe**) exhibited maximum activity with percentage inhibition of 58.12 ± 0.32 which has been followed by compound **VIc** with percentage inhibition of 51.75 ± 0.38. The compounds **VIb** and **VIa** were found to be next in the order with percentage inhibition of 38.42 ± 0.26 and 25.68 ± 0.34. The compound **VId** has shown least activity with percentage inhibition of 20.12 ± 0.13.

Table. 4: Anti-inflammatory activity of synthesized derivatives. (VIa - VIe).

Compound 50mg/kg	Dose (mg/kg)	% Inhibition
VIa	50	25.68 ± 0.34
VIb	50	38.42 ± 0.26
VIc	50	51.75 ± 0.38
VId	50	20.12 ± 0.13
VIe	50	58.12 ± 0.32
Diclofenac sodium	20	85.26 ± 0.45

Values (edema inhibition) represent mean values ± SD, n = 6.

CONCLUSION

The proposed isatin derivatives were synthesized successfully. All the compounds were evaluated for antibacterial, antifungal and anti-inflammatory activity. All the synthesized compounds were found to have good activity, among all the active compounds of isatin derivatives, compound **VIe** has shown good activity against both the Gram-positive and Gram-negative bacteria. The compound **VIe** has also shown significant antifungal activity with maximum zone of inhibition (13.8 mm) against *A.flavus* and zone of inhibition of 12.9 mm and 11.5 mm against *F.oxysporium* and *P.notatum* and compound also showed high antiinflammatory activity than all the other synthesized compounds with percentage inhibition of 58.12 ± 0.32.

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